Molecular Characterization and Proposed Taxonomic Placement of the Biosimulant 'BG'

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INTRODUCTION

- A brief history of the biosimulant "BG"

    - Migula describes *Bacillus globigii*, 1900

    - Smith reclassifies, *B. subtilis* var. *niger*, 1952

    - Gordon eliminates variation designation, 1973

    - Nakamura proposes *B. atrophaeus* sp. nov., 1989

    - Fritze and Pukall reclassify ATCC 9372, 2001

INTRODUCTION

- ATCC 9372 used as a "surrogate" of *B. anthracis* by the Dugway Proving Ground
- Ambiguous status of other pigmented *Bacillus* strains

INTRODUCTION

- Amplified Fragment Length Polymorphism (AFLP)

    - high resolution genotyping technique

    - use in bacterial taxonomic studies

INTRODUCTION

Restriction digest, EcoRI, MseI

Adaptor ligation

EcoRI fragments   EcoRI/MseI fragments   MseI fragments

Selective amplification, EcoRI-primer*,
MseI-primer

*

Capillary Electrophoresis

METHODS

- 16S rRNA gene sequencing

  - MicroSeq® 500 (Applied Biosystems)

- AFLP

  - 16 selective primer sets

  - Internal size standard

  - Data analysis w/ GelCompar II (Applied Maths)

METHODS

- AFLP

  - EcoRI +C/MseI +CC, EcoRI +C/MseI +CA

  - Reproducibility and baseline variability

  - Removal of ambiguous band classes

  - Creation of a composite data set of Dice coefficients

  - UPGMA dendrogram constructed

  - Bootstrap analysis

METHODS
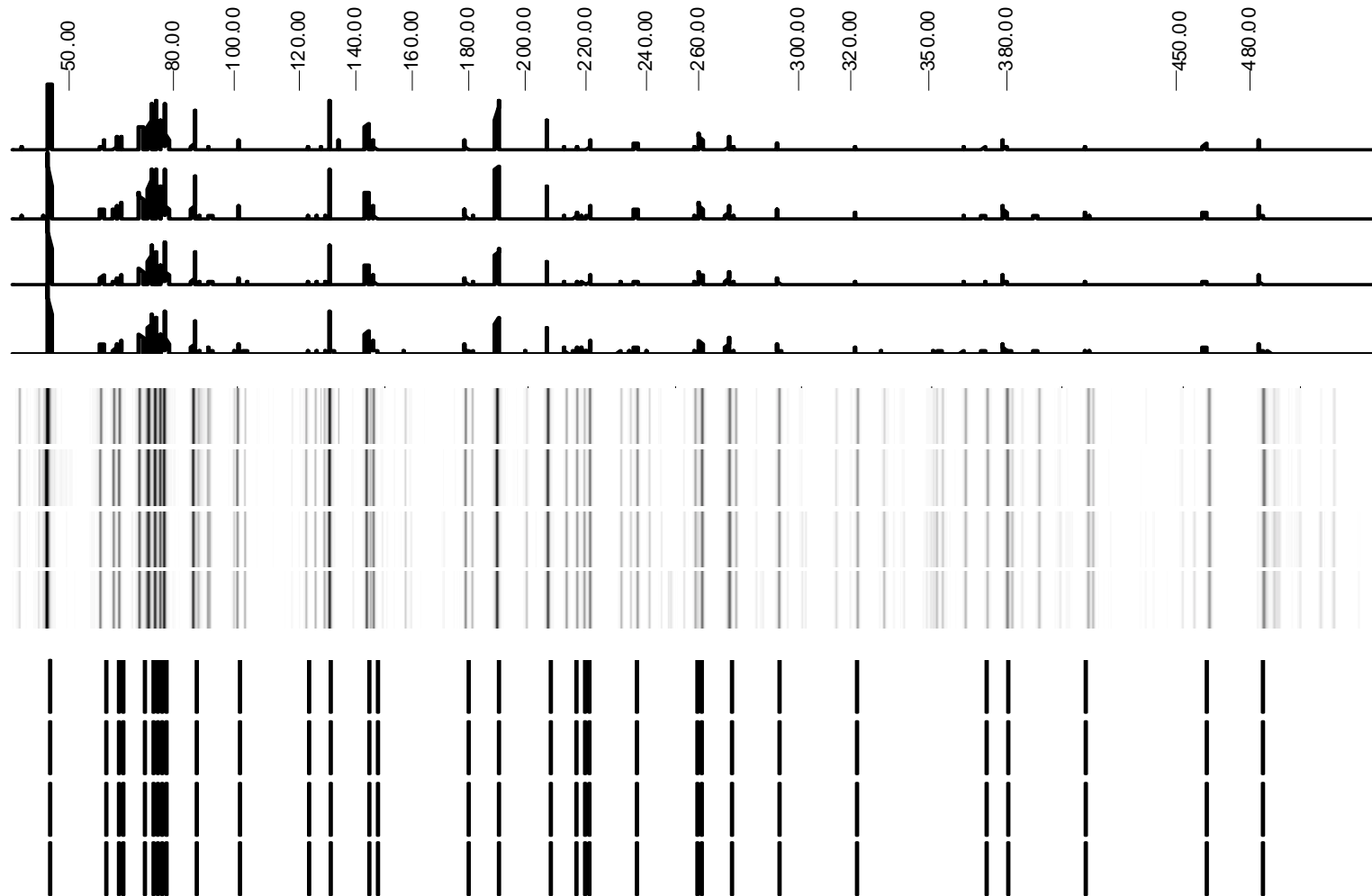
RESULTS

- 16S rRNA gene sequencing   - ATCC 49337 vs 9372
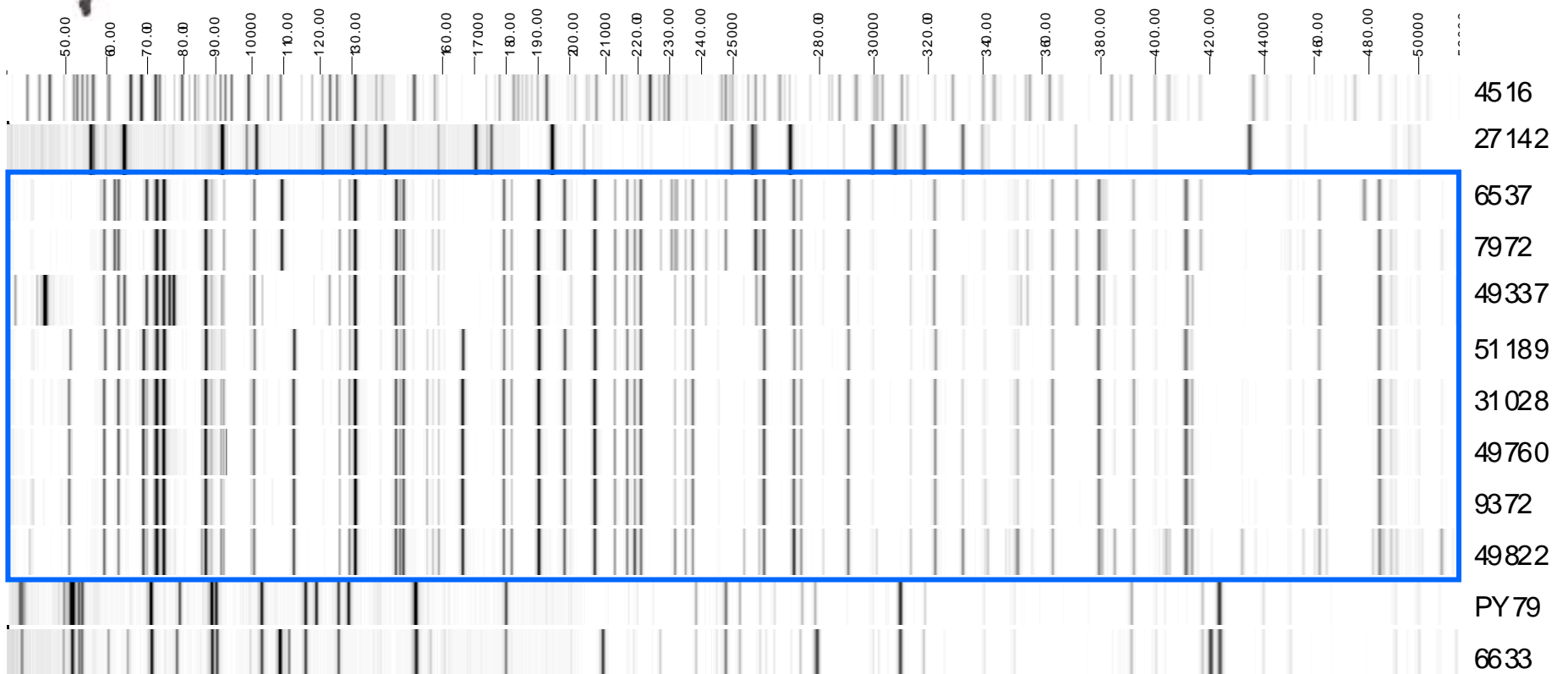
RESULTS

- 16S rRNA gene sequencing

  - *B. atrophaeus* vs *B. subtilis*

    - 99.3% identity (10 base substitutions in ~1500 bases)

RESULTS

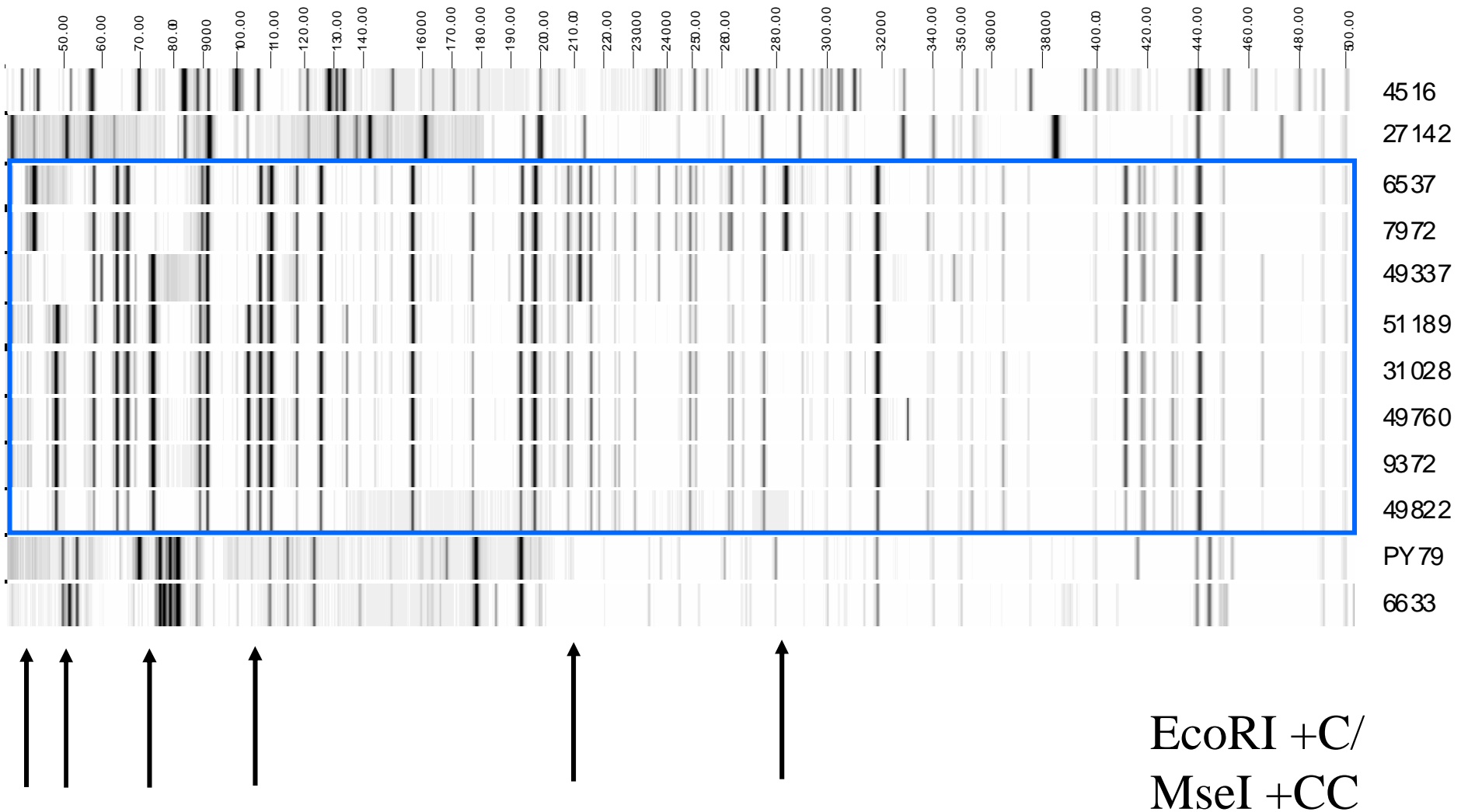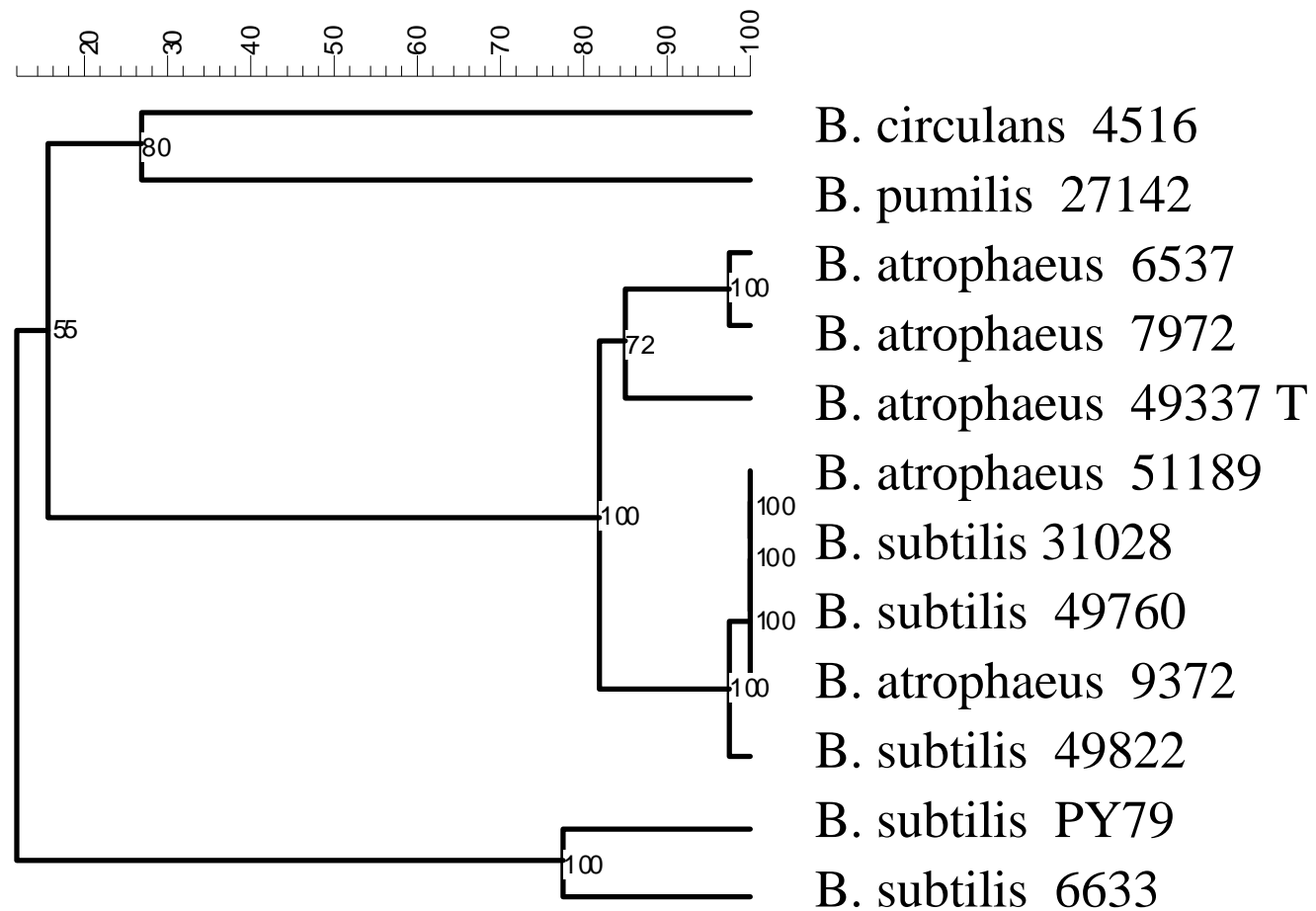

EcoRI +C/
MseI + CA

RESULTS

AFLP-CCC

EcoRI +C/
MseI +CC

RESULTS

- AFLP

    - total number of fragments = 164

    - total among pigmented Bacilli = 72

        - 41 common fragments

        - 31 polymorphisms

RESULTS

SUMMARY

- AFLP data were useful in discriminating between closely related taxa of Bacillus

- Species level variation was easily detected with AFLP

- A distinct cluster of *B. atrophaeus* isolates was revealed

- AFLP data indicate a clear difference between *B. atrophaeus* type strain and Dugway "BG"

- A subspecies of *B. atrophaeus* is proposed; *B. atrophaeus* subsp. *globigii*

- Dugway "BG" is *B. atrophaeus* subsp. *globigii*